Hyperlipidemic Mice Present Enhanced Catabolism and Higher Mitochondrial ATP-Sensitive K^+ Channel Activity

LUCIANE C. ALBERICI,* HELENA C. F. OLIVEIRA,‡ PATRÍCIA R. PATRÍCIO,‡ ALICIA J. KOWALTOWSKI,§ and ANIBAL E. VERCESI*

*Departamento de Patologia Clínica, Faculdade de Ciências Médicas, and ‡Departamento de Fisiologia e Biofísica, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo; and §Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil

Background & Aims: Changes in mitochondrial energy metabolism promoted by uncoupling proteins (UCPs) are often found in metabolic disorders. We have recently shown that hypertriglyceridemic (HTG) mice present higher mitochondrial resting respiration unrelated to UCPs. Here, we disclose the underlying mechanism and consequences, in tissue and whole body metabolism, of this mitochondrial response to hyperlipidemia. **Methods:** Oxidative metabolism and its response to mitochondrial adenosine triphosphate (ATP)-sensitive K^+ channel ($mitoK_{ATP}$) agonists and antagonists were measured in isolated mitochondria, livers, and mice. **Results:** Mitochondria isolated from the livers of HTG mice presented enhanced respiratory rates compared with those from wild-type mice. Changes in oxygen consumption were sensitive to adenosine triphosphate (ATP), diazoxide, and 5-hydroxydecanoate, indicating they are attributable to mitochondrial ATP-sensitive K^+ channel
($mitoK_{ATP}$) activity. Indeed, mitochondria from HTG mice presented enhanced swelling in the presence of K^+ ions, sensitive to $mitoK_{ATP}$ agonists and antagonists. Furthermore, mitochondrial binding to fluorescent glibenclamide indicates that HTG mice expressed higher quantities of $mitoK_{ATP}$. The higher content and activity of liver $mitoK_{ATP}$ resulted in a faster metabolic state, as evidenced by increased liver oxygen consumption and higher body CO_2 release and temperature in these mice. In agreement with higher metabolic rates, food ingestion was significantly larger in HTG mice, without enhanced weight gain. **Conclusions:** These results show that primary hyperlipidemia leads to an elevation in liver $mitoK_{ATP}$ activity, which may represent a regulated adaptation to oxidize excess fatty acids in HTG mice. Furthermore, our data indicate that $mitoK_{ATP}$, in addition to UCPs, may be involved in the control of energy metabolism and body weight.

High plasma levels of triglycerides and free fatty acids can occur due to primary inherited disorders or secondarily to other metabolic diseases such as diabetes and the metabolic syndrome, which also includes insulin resistance, obesity, and hypertension.[1] In conjunction with these disorders, or even individually, hypertriglyceridemia is a risk factor for coronary heart disease, stroke, and nonalcoholic fatty liver disease.[1–3]

A group of proteins that plays a role in the pathogenesis or consequences of metabolic diseases is the uncoupling proteins (UCPs).[4–6] These proteins act as mitochondrial inner membrane fatty acid anion transporters and are widely distributed in many mammalian organs.[7] Because of the proton gradient and free permeability of protonated fatty acids across the inner membrane, a result of UCP activity is mitochondrial uncoupling, including increased resting respiration and decreased membrane potentials and oxidative phosphorylation efficiency.[7] Interestingly, UCP expression is altered by obesity and diabetes[8–10] and in some conditions where circulating lipid levels are modified by hormones,[11] dietary fat,[12] and intravenous heparin plus lipid infusion.[13]

All these conditions present a complex metabolic context where it is difficult to discriminate the role of hyperlipidemia. To study the effects of elevated plasma lipid levels per se, without other metabolic confounding factors, we used mice overexpressing the apolipoprotein CIII, which develop severe hypertriglyceridemia and high plasma levels of free fatty acids[14] but retain normal glucose homeostasis.[15,16] The increased apolipoprotein CIII content in the surface of triglyceride-rich lipoproteins hampers their recognition by specific liver receptors, thus increasing their half-life and free fatty acid release in the plasma compartment.[17] We found that liver mitochondria from these mice presented higher resting respiratory rates.[18] This increase in respiration was not related to the activity of UCPs because (1) the effect was present even in media in which free fatty acids were quenched by bovine serum albumin and (2) uncoupling was not eliminated by the UCP inhibitor GDP (guanosine 5′-diphosphate).[18]

To uncover the cause of this mitochondrial uncoupling in hypertriglyceridemic mice, we now focus our attention on another recently described mild mitochondrial uncoupling pathway: the activity of adenosine triphosphate–sensitive K^+ channels ($mitoK_{ATP}$).[19,20] These inner membrane uniporters promote K^+ influx in a manner counteracted by the K^+/H^+ antiporter (see Figure 1 and Garlid and Paucek[19] for review). The resulting uptake of H^+ through the antiporter decreases the efficiency of oxidative phosphorylation. Uncoupling is limited by K^+ transport rates of $mitoK_{ATP}$, which are quite slow and only allow for mild uncoupling. In addition to mild uncoupling, these channels also promote low-amplitude mitochondrial swelling when active due to the uptake of K^+, the counter-ion phosphate, and water.[21,22] We found strong evidence that the activity and quantity of $mitoK_{ATP}$ channels is augmented in hypertriglyceridemic

Abbreviations used in this paper: DZX, diazoxide; EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; GLY, glyburide/glibenclamide; 5-HD, 5-hydroxydecanoate; HTG, hypertriglyceridemic; $mitoK_{ATP}$, mitochondrial adenosine triphosphate–sensitive K^+ channels; UCP, uncoupling protein; WT, wild-type.

0016-5085/06/$32.00
doi:10.1053/j.gastro.2006.07.021

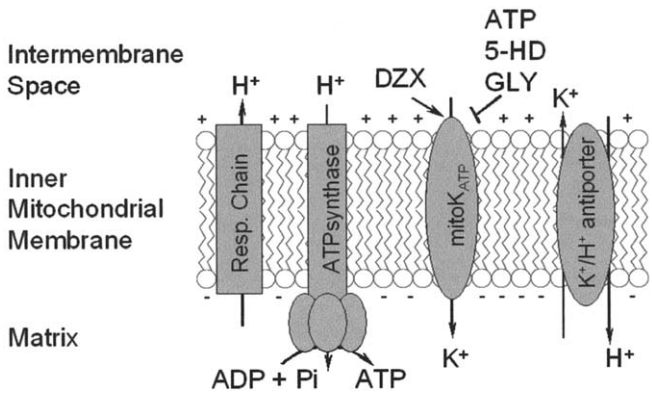

Figure 1. Mitochondrial inner membrane K^+ transport. The mitochondrial respiratory chain generates a proton gradient used by ATP synthase to promote oxidative phosphorylation. MitoK_{ATP} mediates K^+ transport into the matrix in a manner inhibited by ATP, GLY, or 5-HD and stimulated by DZX. K^+ is removed from the mitochondrial matrix in exchange for H^+ by the K^+/H^+ antiporter, resulting in mild uncoupling.

(HTG) mice, providing a mechanistic explanation for the observed uncoupling. In addition, we show evidence that this uncoupling leads to increases in overall respiratory rates and catabolism in HTG livers in mice.

Materials and Methods

Animals

Human apolipoprotein CIII transgenic (line 3707)[23] founders were donated by Dr Alan R. Tall (Columbia University, New York, NY) and crossbred with wild-type (WT) C57Bl6 mice. The apolipoprotein CIII transgenic colony has been kept for 10 years at the animal facilities of the Department of Physiology and Biophysics at the State University of Campinas (Campinas, Brazil). The experiments were approved by the university's ethics committee and are in accordance with the Guidelines for Handling and Training of Laboratory Animals published by the University's Federation for Animal Welfare. Mice had access to standard laboratory rodent chow (CR1; Nuvital, Colombo, Paraná, Brazil) and water ad libitum and were housed at 22°C ± 2°C on a 12-hour light-dark cycle. Male and female heterozygous apolipoprotein CIII transgenic (HTG) and nontransgenic (WT) littermates, aged 4–6 months, were used in this study. Total cholesterol and triglyceride (Chod-Pap; Roche Diagnostic GmbH, Mannheim, Germany) and plasma free fatty acid (Wako Chemical, Neuss, Germany) levels were determined by enzymatic-colorimetric methods according to the manufacturers' instructions. Transgenic mice presented fasting plasma triglyceride levels >300 mg/dL and WT mice levels <100 mg/dL. Four groups (n = 12) of male and female transgenic and WT mice had their body weight gain and food ingestion followed up from weaning (30 days of age) to 6 months of age. Mice and ingested food were weighed 3 times a week. Body weights were taken individually, whereas food ingestion was measured as the average consumed by 4 mice per cage per day. Two groups of transgenic mice (n = 6) were treated with insulin or saline as previously described.[24] Briefly, mice received daily subcutaneous injections of increasing doses of NPH insulin (0.14–1.63 U/30 g body wt, Iolin; Eli Lilly, Indianapolis, IN) or the same volume of saline solution for 7 days. Two thirds of the dose was given at 8 PM and one third at 8 AM. To prevent hypoglycemia, these mice had free access to sugar cubes in addition to the chow diet, and a 5% glucose solution was the only drinking solution offered.

Isolation of Mouse Liver Mitochondria

Mitochondria were isolated by conventional differential centrifugation[25] at 4°C. No differences between sexes of the animals were noted in isolated mitochondrial studies, so analyzed samples from male and female animals were pooled. A liver homogenate was prepared in 250 mmol/L sucrose, 1 mmol/L ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA), 10 mmol/L HEPES buffer (pH 7.2), and 0.01% bovine serum albumin and centrifuged at 600*g* for 10 minutes. The supernatant was recentrifuged at 7000*g* for 10 minutes. The pellet was washed in the same medium devoid of bovine serum albumin and containing 0.1 mmol/L EGTA. The final mitochondrial pellet was diluted in 250 mmol/L sucrose to a protein concentration of 60–80 mg/mL, measured using the Biuret method and bovine serum albumin as the protein standard. Isolated mitochondria were kept over ice and used within 90 minutes of preparation to ensure mitoK_{ATP} activity. Mitochondria isolated in this manner lose matrix K^+ and contract due to low levels of this ion in the isolation buffer and recover K^+ when suspended in K^+-rich buffers.[21,22]

Mitochondrial and Respiratory Rates

Oxygen consumption was measured using a temperature-controlled computer-interfaced Clark-type oxygen electrode from Hansatech Instruments Ltd. (King's Lynn, Norfolk, England) equipped with magnetic stirring at 28°C.

Mitochondrial Swelling

Mitochondrial swelling was estimated from the decrease in absorbance of the mitochondrial suspension measured at 520 nm using a temperature-controlled SLM Aminco DW 2000 spectrophotometer (SLM Instruments, Inc., Urbana, IL) equipped with continuous stirring at 37°C. Swelling rates of freshly isolated mitochondria were measured soon after their addition of K^+-rich, hyposmotic buffers. This procedure allows for a magnified measurement of K^+ uptake rates due to prior K^+ depletion during the mitochondrial isolation procedure.[22]

Liver Respiratory Rates

Mouse livers were rapidly dissected and chopped into 1-mm cubes using a tissue chopper. Approximately 50-mg liver samples were incubated in 1 mL Krebs–Henseleit solution (37°C) containing 10 mmol/L glucose. Oxygen consumption was measured using a Clark-type electrode as described previously. The exact protein content of each homogenized tissue sample was then determined using the Biuret method, and respiratory rates were calculated.

CO_2 Production Rates In Vivo

CO_2 production in vivo was measured in a temperature-monitored respirometer described by Calegario et al.[26] Fed mice weighing between 24 and 28 g were adapted to the respirometer chamber twice a day for 5 minutes for 5 days. After the adaptation period, CO_2 expiration of each mouse was monitored for 5 minutes once a day, between 9 AM and 11 AM, for 5 consec-

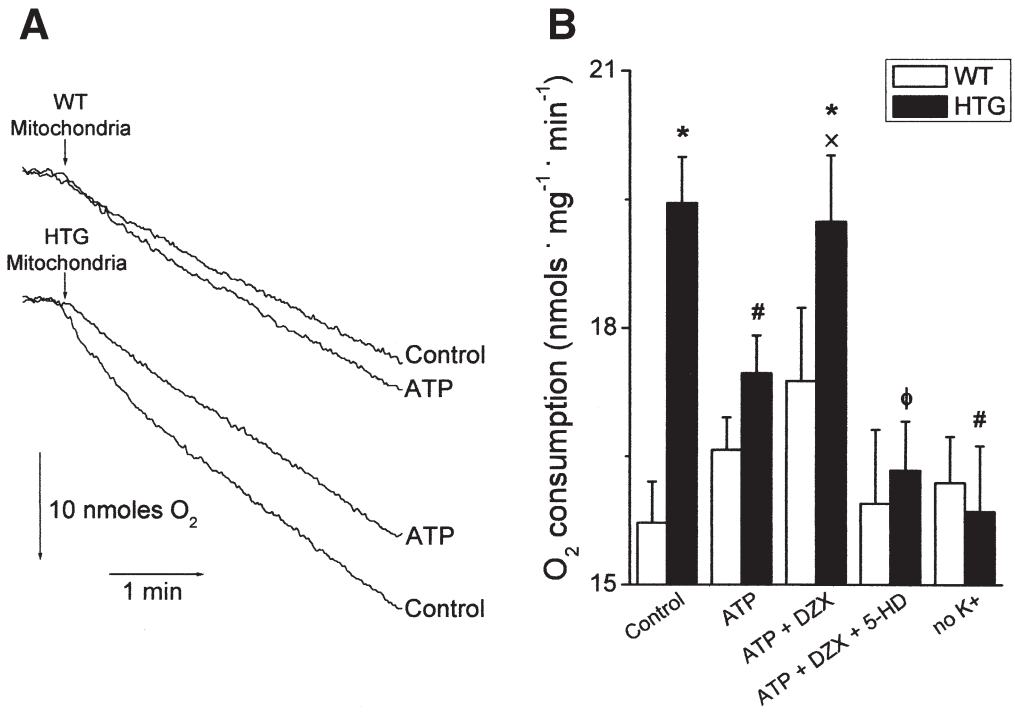

Figure 2. Enhanced resting respiration in HTG mitochondria is due to ATP-sensitive K^+ uptake. Typical traces are shown in *A*, and averages ± SEM are depicted in *B*. WT and HTG mitochondria (0.5 mg/mL) were added to 28°C, pH 7.2 (KOH) medium containing 125 mmol/L sucrose, 65 mmol/L KCl, 10 mmol/L HEPES, 2 mmol/L P_i, 1 mmol/L Mg^{2+}, 0.4 mmol/L EGTA, 4 mmol/L succinate, and 1 μg/mL oligomycin in the presence of 0.1 mmol/L ATP, 12 μmol/L DZX, and 60 μmol/L 5-HD, as shown. No K^+, experiments conducted in media in which all K^+ salts were substituted by Li-positive salts. $^*P <$ .01 vs WT under control conditions; $^{\#}P <$ .01 vs HTG under control conditions; $^{\times}P = .05$ vs HTG in the presence of ATP; $^{\phi}P <$ 05 vs HTG in the presence of ATP plus DZX.

utive days. CO_2 production rates were calculated as averages of 5 measurements for each mouse.

Body Temperature

Rectal temperatures were measured using a digital thermometer (BD Basic; Becton Dickinson and Company, São Paulo, Brazil). Mice were adapted to rapid comfortable immobilization and rectal temperature measurements for 5 days, between 2 PM and 3 PM. Measurements were then conducted for 5 days, during three 20-second periods. Average rectal temperatures for each animal during these measurements were then determined and compared.

Data Analysis

Data shown as traces are representative of at least 3 repetitions using different preparations. Other data are averages ± SEM. Statistical analysis was performed using one-way analysis of variance comparisons (Figures 2–4) and analysis of variance and Student *t* tests (with similar results; Figures 5 and 6) conducted using Origin 7.0 software (OringinLab Corp., Northampton, MA). $P \leq .05$ was considered significant.

Results

Confirming previous results,[18] we found that mitochondria isolated from livers of HTG mice present higher O_2 consumption rates than WT mitochondria when incubated under basal conditions in which no oxidative phosphorylation occurs (see representative traces in Figure 2*A* and averages in Figure 2*B*). In our prior study,[18] no increases in respiratory rates under state III conditions, in which oxidative phosphorylation is stimulated, were observed, indicating these changes are not due to higher maximal respiratory capacity. Instead, we found here that enhanced respiratory rates of HTG mitochondria were significantly prevented by the presence of ATP (Figure 2). Furthermore, the effects of ATP were reversed by the mitoK$_{ATP}$ agonist diazoxide (DZX),[27] strongly suggesting that the increase in respiration is due to K^+ cycling stimulated by the activity of this channel. Indeed, the DZX effect was completely abrogated by the mitoK$_{ATP}$ antagonist 5-hydroxydecanoate (5-HD),[28] and no differences in respiratory rates could be observed in media devoid of K^+ salts. These results indicate that the increased respiratory rates observed in HTG mitochondria are due to the activity of mitoK$_{ATP}$.

To directly assess if ATP-sensitive K^+ transport was enhanced in HTG compared to WT mitochondria, we measured light scattering changes in these suspensions. Mitochondria lose K^+ during isolation, and the uptake of this ion during the first few seconds of incubation in K^+-rich media is enhanced by mitoK$_{ATP}$ activity. Because K^+ uptake is accompanied by phosphate (as a counter-ion) and water, mitochondrial matrix swelling occurs, with concomitant decreases in light scattering of the suspension.[21,22] Swelling experiments are an important complement to the respiratory rate measurements conducted earlier, because DZX is known to have protonophoric effects and leads to respiratory inhibition in mitochondria when used at toxic concentrations.[22] Because the inner membrane potential is a driving force for K^+ uptake,[19] both respiratory inhibition and protonophoric activity decrease mitochondrial swelling, while mitoK$_{ATP}$ activation promoted by low doses of DZX enhances swelling.[22]

We found that swelling in WT mitochondria was poorly inhibited by ATP, indicating low levels of mitoK$_{ATP}$ activity (see Figure 3*A* for typical traces and Figure 3*B* for average swelling rates). On the other hand, swelling rates in HTG mitochondria were significantly larger and prevented by ATP. This difference in swelling rates was only noted in media containing K^+ ions. Indeed, as expected for mitoK$_{ATP}$-mediated swelling, DZX reversed the ATP effect in a manner prevented by mitoK$_{ATP}$

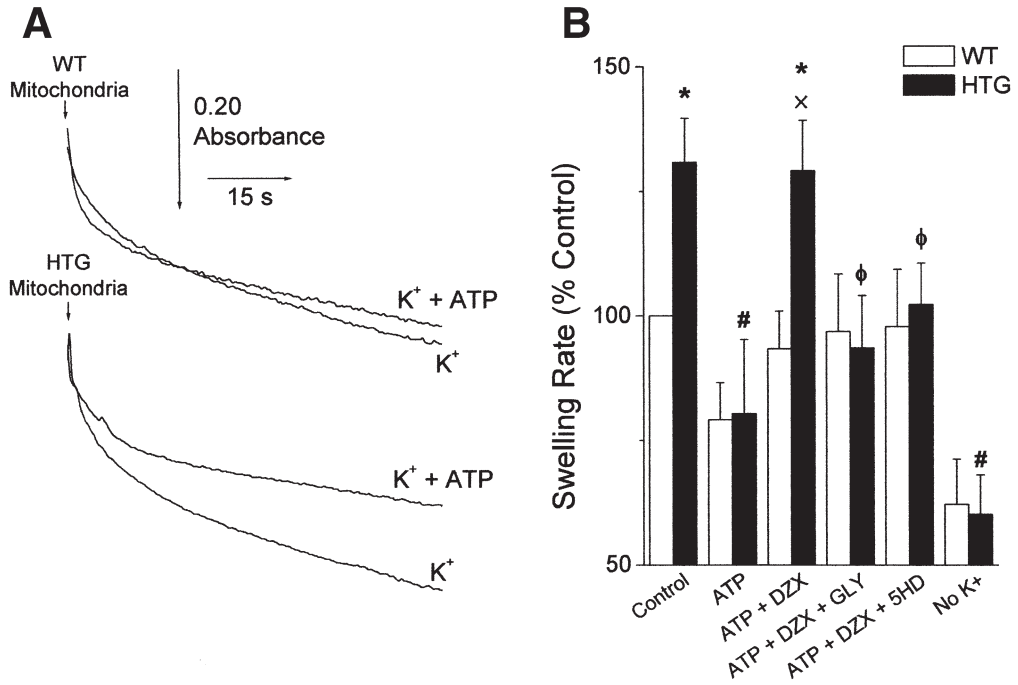

Figure 3. HTG mitochondria present enhanced ATP-sensitive K^+ uptake. Typical traces are shown in *A*, and averages ± SEM of swelling rates during the first 15 seconds are depicted in *B* Mitochondria (0.5 mg/mL) were added to 28°C, pH 7.2 (KOH) medium containing 100 mmol/L KCl, 5 mmol/L HEPES, 2 mmol/L P_i, 1 mmol/L Mg^{2+}, 0.1 mmol/L EGTA, 2 mmol/L succinate, and 1 μg/mL oligomycin in the presence of 0.2 mmol/L ATP, 30 μmol/L DZX, 10 μmol/L GLY, and 60 μmol/L 5-HD as indicated. No K^+, experiments conducted in media in which all K^+ salts were substituted by Li-positive salts. $^*P < .05$ vs WT under control conditions; $^{\#}P < .05$ vs HTG under control conditions; $^{\times}P < 05$ vs HTG in the presence of ATP; $^{\phi}P \leq .05$ vs HTG in the presence of ATP plus DZX.

antagonists glibenclamide (glyburide [GLY]) and 5-HD. Based on these findings, we conclude that mitoK$_{ATP}$ activity is enhanced in the livers of HTG mice.

Next, we assessed if larger quantities of mitoK$_{ATP}$ were present in HTG mitochondria compared with WT. Because the molecular identity of mitoK$_{ATP}$ is a matter of debate, we compared levels of bound fluorescent GLY (BODIPY FL GLY; Molecular Probes, Eugene, OR) in isolated mitochondrial preparations. This measurement estimates the content of mitochondrial sulfonylurea receptors, a component of mitoK$_{ATP}$.[29] We found that the fluorescent GLY binding to HTG mitochondria was significantly enhanced (110.4% ± 2.4% of WT; $P < .05$), confirming that liver content of mitochondrial sulfonylurea receptors is higher in HTG mice.

The activation of mitoK$_{ATP}$ in HTG mice could be a consequence of higher circulating or intracellular lipids. We showed previously[18] that fibrate treatment, which decreases plasma triglyceride levels and accelerates intracellular fatty acid beta oxidation, normalized mitochondrial respiration in these HTG mice. To further check the specific role of intracellular free fatty acid content, we treated HTG mice with increasing doses of insulin[24] during a 7-day period, a protocol that also decreases

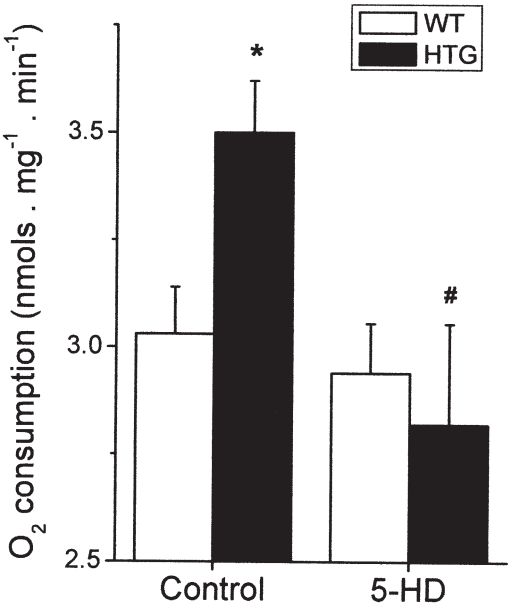

Figure 4. MitoK$_{ATP}$ activity increases respiratory rates in HTG livers. Data shown are average ± SEM respiratory rates of liver fragments incubated at 37°C in Krebs–Henseleit solution, as described in Materials and Methods. Mice received intraperitoneal saline (control) or 5-HD (10 mg/kg body wt) 1 hour before liver excision. $^*P < .05$ vs WT under control conditions; $^{\#}P < .05$ vs HTG under control conditions.

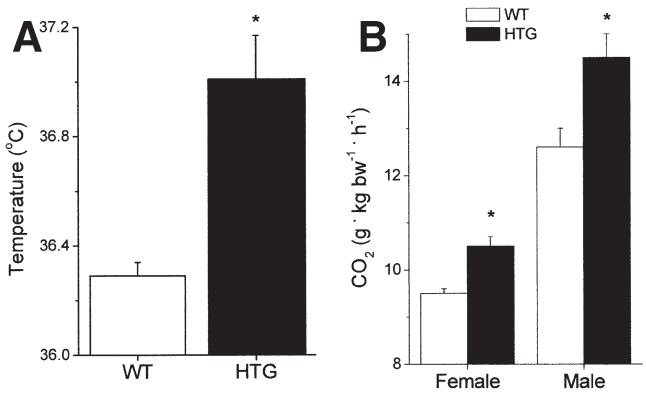

Figure 5. Body temperature and CO_2 production is enhanced in HTG mice. (*A*) Rectal body temperatures and (*B*) CO_2 release rates were compared in WT and HTG mice, as described in Materials and Methods. $^*P < .05$ vs WT.

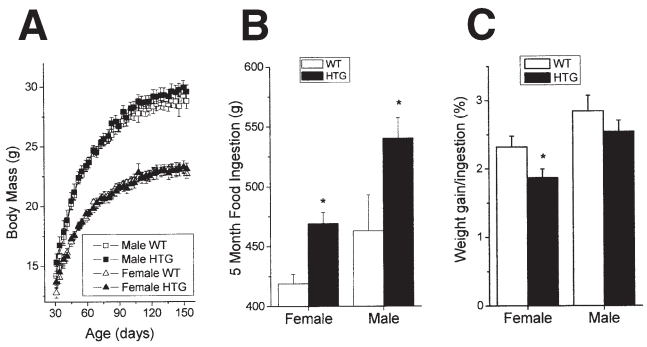

Figure 6. Food ingestion is enhanced in HTG mice. (*A*) Body weight, (*B*) food ingestion, and (*C*) the efficiency of the conversion of ingested food in HTG and WT mice were measured from weaning (1 month) to 6 months of age, as described in Materials and Methods. Data represent means ± SEM (in grams). *$P < .05$ vs WT.

plasma lipid levels but stimulates intracellular fatty acid synthesis. Indeed, in vivo insulin treatment reduced triglyceride levels by 30% (340 ± 56 vs 481 ± 91 mg/dL, respectively, for insulin- and saline-treated HTG mice; $P < .05$). However, insulin did not correct higher resting respiration in HTG liver mitochondria (22.4 ± 2.5 vs 21.8 ± 1.5 nmol oxygen · mg protein^{-1} · min^{-1}, respectively, for insulin- and saline-treated HTG mice). Thus, both fibrates and insulin correct plasmatic triglyceride levels but present opposite effects on intracellular fatty acid metabolism, while fibrates, but not insulin, reverse the respiratory increments observed in these animals. As a result, we conclude that higher respiratory rates observed in HTG mitochondria are related to changes in intracellular fatty acid metabolism observed in HTG mice but not enhanced plasmatic lipid contents.

Mild levels of uncoupling promoted by enhanced mitoK$_{ATP}$ expression and activity in mitochondria isolated from HTG mice could result in changes in overall liver metabolism. To check this possibility, we measured oxygen consumption in liver fragments (Figure 4). HTG liver oxygen consumption rates were about 16% greater than those of WT livers. This increase is lower than the 24% increase in resting respiratory rates observed in mitochondria isolated from these livers (see Figure 2), probably because intact liver samples conduct oxidative phosphorylation and thus are not in resting state. The increment in respiratory rates in intact livers could nonetheless be ascribed to mitoK$_{ATP}$ activity, because 5-HD injected 1 hour before liver excision significantly decreased O_2 consumption only in HTG liver fragments and made respiratory rates indistinguishable from those of WT livers (Figure 4).

Because the liver has an important role in overall oxidative metabolism and nonshivering thermogenesis,[30] we evaluated whether HTG mice present different metabolic rates compared with WT mice. As shown in Figure 5*A*, body temperatures in HTG mice were significantly augmented, a finding that could indicate a hypermetabolic state in these mice. Indeed, CO_2 production rates by both female and male HTG mice were significantly higher than WT mice (Figure 5*B*).

If HTG mice present a higher metabolic rate, they should be leaner or present higher food ingestion than WT mice. Thus, we followed up body weight gain and food ingestion in these mice from weaning up to 6 months of age. As shown in Figure 6, although body weight gain along time is similar, the amount of food ingested is significantly greater for female and male HTG than WT mice and efficiency of the conversion of ingested food is reduced in HTG mice, especially in HTG female mice, confirming higher energy dissipation in HTG mice.

Discussion

Recently, much attention has been focused on correlations between changes in mitochondrial energy metabolism and pathologic conditions found in metabolic disorders. Altered mitochondrial energy metabolism has been shown to be both cause and consequence of these conditions. For example, a mutation in mitochondrial DNA encoding a transfer RNA causes maternally inherited hypomagnesemia, hypertension, and hypercholesterolemia.[31] Certain mitochondrial DNA polymorphisms are related to insulin resistance and can be predictors of the development of diabetes.[32] Furthermore, UCP expression has been shown to change not only in diabetes[9,10] but also with altered dietary and metabolic conditions. These changes may be related to body mass index,[9] insulin resistance,[12] or circulating free fatty acids.[8] Although UCP activity mediates energy dissipation, favoring a catabolic state, the effect of UCPs on the control of body weight is uncertain. While mice overexpressing human UCP3 in skeletal muscle are hyperphagic and lean,[33] knockout mice in which UCP3 is absent are not obese.[34,35]

We found that normal-weight, glucose-tolerant, and HTG mice present enhanced liver mitochondrial inner membrane proton conductance (Figure 2 and Alberici et al[18]). However, we could not find evidence that this uncoupling was related to UCP activity.[18] Thus, to explain the uncoupling observed in HTG mice, we focused our attention on another regulated mitochondrial uncoupling pathway: the concerted activity of mitoK$_{ATP}$ and the K^+/H^+ exchanger (see Figure 1). Potassium uptake into the mitochondrial matrix through mitoK$_{ATP}$ channels is accompanied by phosphate and water, resulting in matrix swelling. This swelling activates K^+/H^+ antiporters,[19] and the net result is the entrance of a proton for every K^+ exchanged, resulting in uncoupling.

Using 3 different techniques, mitochondrial oxygen consumption measurements (Figure 1), light scattering estimates of mitochondrial swelling (Figure 2), and binding to fluorescent GLY (data in text), we found that mitoK$_{ATP}$ activity and expression is augmented in the livers of HTG mice. This enhanced mitoK$_{ATP}$ activity increases mitochondrial respiratory rates under nonphosphorylating conditions (Figure 2) and enhances K^+ uptake and matrix volumes relative to control mitochondria (Figure 3). Indeed, these respiratory and volume effects are inhibited by mitoK$_{ATP}$ antagonists ATP, GLY, and 5-HD (Figures 2 and 3). The lack of observable uncoupling and swelling in HTG relative to WT mitochondria in media devoid of K^+ ions (Figure 2) provides unequivocal evidence that this effect occurs secondarily to enhanced K^+ cycling.

It should be noted that, due to reports from both our group and others[22,28,36,37] (see Facundo et al[20] for a critical review) of toxic effects of drugs such as DZX, 5-HD, and GLY that are unrelated to mitoK$_{ATP}$ activity, we conducted extensive control experiments to ensure our results were not attributable to these toxic effects. These controls include avoiding the use of GLY in respiratory experiments (due to its inhibitory effect at low doses[28]), experiments using only the physiologic mitoK$_{ATP}$ in-

hibitor ATP, experiments conducted in the absence of K^+ salts, comparisons between respiration and swelling effects, and the use of low drug doses, previously shown not to cause changes in inner membrane potentials under conditions similar to ours.[22] Indeed, toxic effects of 5-HD and GLY in our isolated mitochondrial studies are unlikely because their effects were state dependent[28] and not observed in the absence of ATP and DZX (results not shown). Furthermore, toxic effects of DZX (which include respiratory inhibition and protonophoric effects) cannot lead to mitochondrial swelling or alterations in swelling and respiration exclusively in HTG animals. Finally, our results are not observable in media devoid of K^+, confirming they are related to a selective K^+ transport pathway that promotes swelling and increases respiration, such as mitoK_{ATP}.

In addition to presenting increased respiratory rates in isolated mitochondria, HTG mice display enhanced oxygen consumption rates in intact liver tissue in a manner prevented by prior in vivo intraperitoneal administration of the mitoK_{ATP} antagonist 5-HD (Figure 4). Furthermore, HTG mice present elevated overall metabolic rates, as attested by increased body temperature, CO_2 release (Figure 5), and food ingestion (Figure 6). These results suggest that the enhanced activity of mitoK_{ATP} observed in HTG livers may have important overall whole body metabolic consequences. Unfortunately, we were unable to directly test this hypothesis due to the multiple systemic effects of mitoK_{ATP} antagonists unrelated to the activity of this channel. However, we were able to address the probable cause of these changes. Intracellular availability of fatty acids is likely implicated in this adaptative phenomenon, because higher resting respiration in HTG mice was corrected by activation of fatty acid beta oxidation promoted by fibrate treatment[18] but not by fatty acid oxidation suppression promoted by insulin. While both fibrates and insulin correct hyperlipidemia, they have opposite effects on intracellular fatty acid metabolism. The lack of an insulin effect in mitochondrial K^+-dependent uncoupling differs from previously described mitochondrial alterations found in diabetes, obesity, and the metabolic syndrome, often attributed directly to hyperinsulinemia or insulin resistance.[8–10,12]

In conclusion, we found clear evidence that hyperlipidemia increases mitoK_{ATP} expression and activity. We propose that changes in mitoK_{ATP} activity represent an adaptation that allows HTG mice to oxidize excess intracellular free fatty acids. This suggests that mitoK_{ATP}, in addition to UCPs, is involved in the control of the energy metabolism and body weight.

References

1. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004; 109:433–438.
2. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med 1999;107:450–455.
3. Reid AE. Nonalcoholic steatohepatitis. Gastroenterology 2001; 121:710–723.
4. Jezek P, Garlid KD. Mammalian mitochondrial uncoupling proteins. Int J Biochem Cell Biol 1998;30:1163–1168.
5. Boss O, Muzzin P, Giacobino JP. The uncoupling proteins, a review. Eur J Endocrinol 1998;139:1–9.
6. Brand MD, Esteves TC. Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. Cell Metab 2005;2: 85–93.
7. Garlid KD, Jaburek M, Jezek P, Varecha M. How do uncoupling proteins uncouple? Biochim Biophys Acta 2000;1459:383–389.
8. Boss O, Bobbioni-Harsch E, Assimacopoulos-Jeannet F, Muzzin P, Munger R, Giacobino JP, Golay A. Uncoupling protein-3 expression in skeletal muscle and free fatty acids in obesity. Lancet 1998;351:1933.
9. Bao S, Kennedy A, Wojciechowski B, Wallace P, Ganaway E, Garvey WT. Expression of mRNAs encoding uncoupling proteins in human skeletal muscle: effects of obesity and diabetes. Diabetes 1998;47:1935–1940.
10. Hidaka S, Yoshimatsu H, Kakuma T, Sakino H, Kondou S, Hanada R, Oka K, Teshima Y, Kurokawa M, Sakata T. Tissue-specific expression of the uncoupling protein family in streptozotocin-induced diabetic rats. Proc Soc Exp Biol Med 2000;224: 172–177.
11. Gong DW, He Y, Karas M, Reitman M. Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, beta3-adrenergic agonists, and leptin. J Biol Chem 1997;272:24129–24132.
12. Samec S, Seydoux J, Dulloo AG. Post-starvation gene expression of skeletal muscle uncoupling protein 2 and uncoupling protein 3 in response to dietary fat levels and fatty acid composition: a link with insulin resistance. Diabetes 1999;48:436–441.
13. Nisoli E, Carruba MO, Tonello C, Macor C, Federspil G, Vettor R. Induction of fatty acid translocase/CD36, peroxisome proliferator-activated receptor-gamma2, leptin, uncoupling proteins 2 and 3, and tumor necrosis factor-alpha gene expression in human subcutaneous fat by lipid infusion. Diabetes 2000;49:319–324.
14. Ito Y, Azrolan N, O'Connell A, Walsh A, Breslow JL. Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. Science 1990;249:790–793.
15. Reaven GM, Mondon CE, Chen YD, Breslow JL. Hypertriglyceridemic mice transgenic for the human apolipoprotein C-III gene are neither insulin resistant nor hyperinsulinemic. J Lipid Res 1994; 35:820–824.
16. Amaral MEC, Oliveira HCF, Carneiro EM, Delghingaro-Augusto V, Vieira E, Berti JA, Boschero AC. Plasma glucose regulation and insulin secretion in hypertriglyceridemic mice. Horm Metab Res 2002;34:21–26.
17. Aalto-Setala K, Fisher EA, Chen X, Chajek-Shaul T, Hayek T, Zechner R, Walsh A, Ramakrishnan R, Ginsberg HN, Breslow JL. Mechanism of hypertriglyceridemia in human apolipoprotein (apo) CIII transgenic mice. Diminished very low density lipoprotein fractional catabolic rate associated with increased apo CIII and reduced apo E on the particles. J Clin Invest 1992;90:1889–900.
18. Alberici LC, Oliveira HC, Bighetti EJ, de Faria EC, Degaspari GR, Souza CT, Vercesi AE. Hypertriglyceridemia increases mitochondrial resting respiration and susceptibility to permeability transition. J Bioenerg Biomembr 2003;35:451–457.
19. Garlid KD, Paucek P. Mitochondrial potassium transport: the K^+ cycle. Biochim Biophys Acta 2003;1606:23–41.
20. Facundo HT, Fornazari M, Kowaltowski AJ. Tissue protection mediated by mitochondrial K^+ channels. Biochim Biophys Acta 2006;40:469–479.
21. Beavis AD, Lu Y, Garlid KD. On the regulation of K^+ uniport in intact mitochondria by adenine nucleotides and nucleotide analogs. J Biol Chem 1993;268:997–1004.
22. Kowaltowski AJ, Seetharaman S, Paucek P, Garlid KD. Bioenergetic consequences of opening the ATP-sensitive K^+ channel of heart mitochondria. Am J Physiol Heart Circ Physiol 2001;280: H649–H657.
23. Walsh A, Azrolan N, Wang K, Marcigliano A, O'Connell A, Breslow JL. Intestinal expression of the human apoA-I gene in transgenic

mice is controlled by a DNA region 3′ to the gene in the promoter of the adjacent convergently transcribed apoC-III gene. J Lipid Res 1993;34:617–623.
24. Berti JA, Casquero AC, Patricio PR, Bighetti EJB, Carneiro EM, Boschero AC, Oliveira HCF. Cholesteryl ester transfer protein expression is down-regulated in hyperinsulinemic transgenic mice. J Lipid Res 2003;44:1870–1876.
25. Kaplan RS, Pedersen PL. Characterization of phosphate efflux pathways in rat liver mitochondria. Biochem J 1983;212:279–288.
26. Calegario FF, Cosso RG, Fagian MM, Almeida FV, Jardim WF, Jezek P, Arruda P, Vercesi AE. Stimulation of potato tuber respiration by cold stress is associated with an increased capacity of both plant uncoupling mitochondrial protein (PUMP) and alternative oxidase. J Bioenerg Biomembr 2003;35:211–220.
27. Garlid KD, Paucek P, Yarov-Yarovoy V, Sun X, Schindler PA. The mitochondrial K_{ATP} channel as a receptor for potassium channel openers. J Biol Chem 1996;271:8796–8799.
28. Jaburek M, Yarov-Yarovoy V, Paucek P, Garlid KD. State-dependent inhibition of the mitochondrial K_{ATP} channel by glyburide and 5-hydroxydecanoate. J Biol Chem 1998;273:13578–13582.
29. Bajgar R, Seetharaman S, Kowaltowski AJ, Garlid KD, Paucek P. Identification and properties of a novel intracellular (mitochondrial) ATP-sensitive potassium channel in brain. J Biol Chem 2001;276:33369–33374.
30. Rolfe FS, Brown GC. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiol Rev 1997;77:731–758.
31. Wilson FH, Hariri A, Farhi A, Zhao H, Petersen KF, Toka HR, Nelson-Williams C, Raja KM, Kashgarian M, Shulman GI, Scheinman SJ, Lifton RP. A cluster of metabolic defects caused by mutation in a mitochondrial tRNA. Science 2004;306:1190–1194.
32. Lee HK, Park KS, Cho YM, Lee YY, Pak YK. Mitochondria-based model for fetal origin of adult disease and insulin resistance. Ann N Y Acad Sci 2005;1042:1–18.
33. Clapham JC, Arch JR, Chapman H, Haynes A, Lister C, Moore GB, Piercy V, Carter SA, Lehner I, Smith SA, Beeley LJ, Godden RJ, Herrity N, Skehel M, Changani KK, Hockings PD, Reid DG, Squires SM, Hatcher J, Trail B, Latcham J, Rastan S, Harper AJ, Cadenas S, Buckingham JA, Brand MD, Abuin A. Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean. Nature 2000;406:415–418.
34. Gong DW, Monemdjou S, Gavrilova O, Leon LR, Marcus-Samuels B, Chou CJ, Everett C, Kozak LP, Li C, Deng C, Harper ME, Reitman ML. Lack of obesity and normal response to fasting and thyroid hormone in mice lacking uncoupling protein-3. J Biol Chem 2000;275:16251–16257.
35. Vidal-Puig AJ, Grujic D, Zhang CY, Hagen T, Boss O, Ido Y, Szczepanik A, Wade J, Mootha V, Cortright R, Muoio DM, Lowell BB. Energy metabolism in uncoupling protein 3 gene knockout mice. J Biol Chem 2000;275:16258–16266.
36. Holmuhamedov EL, Jahangir A, Oberlin A, Komarov A, Colombini M, Terzic A. Potassium channel openers are uncoupling protonophores: implication in cardioprotection. FEBS Lett 2004;568:167–170.
37. Minners J, McLeod CJ, Sack MN. Mitochondrial plasticity in classical ischemic preconditioning-moving beyond the mitochondrial K_{ATP} channel. Cardiovasc Res 2003;59:1–6.

Received February 24, 2006. Accepted June 21, 2006.

Address requests for reprints to: Anibal E. Vercesi, MD, PhD, Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, 13083-970, Campinas, São Paulo, Brazil. e-mail: anibal@unicamp.br; fax: (55) 19-37887414.

Supported by grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). L.C.A. and P.R.P. are supported by FAPESP and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), respectively.